

IN THE CLAIMS

Please withdraw claims 1, 5-12 and 23-26. Please amend claims 2-4, 13-22 and 27-31.

1. (withdrawn) A method for site-specific incorporation of derivatized dideoxynucleotides into DNA comprising reacting an archaeon Family B DNA polymerase, a primed DNA template and nucleotide solution containing at least one derivatized dideoxynucleotide to produce fragments of DNA with the derivatized dideoxynucleoside covalently attached to the 3' terminal residue wherein the derivatized dideoxynucleotide is incorporated more efficiently than the corresponding underivatized dideoxynucleotide.

2. (presently amended) A method for site-specific incorporation of acyclonucleotides into DNA, comprising:
reacting an archaeon Family B DNA polymerase with a primed DNA template and nucleotide solution containing at least one acyclonucleotide to produce fragments of DNA with the acyclonucleotide covalently attached to the 3' terminal residue;

wherein the DNA polymerase is encoded by an isolated DNA fragment that hybridizes in a Southern blot to an isolated DNA fragment selected from the group consisting of a DNA fragment having nucleotides 1-1274 of SEQ ID NO:4, a DNA fragment having nucleotides 291-1772 of SEQ ID NO:4, a DNA fragment having nucleotides 3387-3533 of SEQ ID NO:4, a DNA fragment having nucleotides 4704-5396 of SEQ ID NO:4, and a DNA fragment having nucleotides 4718-5437 of SEQ ID NO:4, wherein hybridization is conducted under the following conditions: a) hybridization: 0.75 M NaCl, 0.15 M Tris, 10 mM EDTA, 0.1% sodium pyrophosphate, 0.1% sodium lauryl sulfate, 0.03% BSA, 0.03% Ficoll 400, 0.03% PVP and 100 µg/ml boiled calf thymus

DNA at 50°C for about 12 hours and; b) wash: 3X30 minutes with 0.1X SET, 0.1% SDS, 0.1% sodium pyrophosphate and 0.1 M phosphate buffer at 45°C.

3. (presently amended) A method for site-specific incorporation of derivatized acyclonucleotides into DNA, comprising:
reacting an archaeon Family B DNA polymerase with a primed DNA template and a nucleotide solution containing at least one derivatized acyclonucleotide to produce fragments of DNA with the derivatized acyclonucleotide covalently attached to the 3' terminal residue;
wherein the DNA polymerase has at least 30% primary amino acid sequence identity with Vent DNA polymerase.
4. (presently amended) The method of claims 1 or 2 or 3 wherein the derivative-acyclonucleotide comprises a detection reagent.
5. (withdrawn) The method of claims 1 or 3 where the derivative comprises a dye-label.
6. (withdrawn) The method of claims 1 or 3 where the derivative comprises a dye selected from the group consisting of TAMRA, ROX, R6G, Fluorescein-12, IRD40, IRD700, FI 6-(((4,(4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)phenoxy)acetyl) amino), 6-((4,4-3-dimethyl-5-(methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino), 6-((4,4-difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino)and 6-((4,4-difluoro-5-7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino).

7. (withdrawn) The method of claims 2 or 3 where the acyclonucleotide is radioactively labeled.

8. (withdrawn) The method of claims 1-3 wherein the DNA polymerase has at least about 20% primary amino acid sequence identity with Vent DNA polymerase.

9. (withdrawn) The method of claims 1-3 wherein the DNA polymerase has at least about 30% primary amino acid sequence identity with Vent DNA polymerase.

10. (withdrawn) The method of claims 1-3 wherein the DNA polymerase has at least about 70% primary amino acid sequence identity with Vent DNA polymerase.

11. (withdrawn) The method of claims 1-3 wherein the DNA polymerase binds to an antibody probe that has antigenic specificity to Vent DNA polymerase.

12. (withdrawn) The method of claims 1-3 wherein the DNA polymerase is encoded by an isolated DNA fragment that hybridizes in a Southern blot to an isolated DNA fragment selected from the group consisting of a DNA fragment having nucleotides 1-1274 of SEQ ID NO:4, a DNA fragment having nucleotides 291-1772 of SEQ ID NO:4, a DNA fragment having nucleotides 3387-3533 of SEQ ID NO:4, a DNA fragment having nucleotides 4704-5396 of SEQ ID NO:4, and a DNA fragment having nucleotides 4718-5437 of SEQ ID NO:4, wherein hybridization is conducted under the following conditions: a) hybridization: 0.75 M NaCl, 0.15 M Tris, 10 mM EDTA, 0.1% sodium pyrophosphate, 0.1% sodium lauryl sulfate, 0.03%

BSA, 0.03% Ficoll 400, 0.03% PVP and 100 µg/ml boiled calf thymus DNA at 50°C for about 12 hours and; b) wash: 3X30 minutes with 0.1X SET, 0.1% SDS, 0.1% sodium pyrophosphate and 0.1 M phosphate buffer at 45°C.

13. (presently amended) The method of claims 1-3-2, wherein the DNA polymerase is selected from the group consisting of Vent, Deep Vent, Pfu and 9°N DNA polymerases.

14. (presently amended) The method of claims 1-3-2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue at a site corresponding to A488, L492, A493 and Y499 in Vent polymerase.

15. (presently amended) The method of claims 1-3-2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to A488 in Vent polymerase with L, I, V, F, S or C.

16. (presently amended) The method of claims 1-3-2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to A488 in Vent DNA polymerase with L.

17. (presently amended) The method of claims 1-3-2 or 3, wherein the DNA polymerase has been mutated by substitution of an amino acid residue corresponding to Y499 in Vent DNA polymerase with L.

18. (presently amended) The method of claims 1-3-2 or 3, wherein the DNA polymerase is a mutant selected from the group consisting of Vent (A488L), Vent' (Y499L) and 9°N (A485L) DNA polymerases.

19. (presently amended) The method of claims 2 or 3, wherein the acyclonucleotide is incorporated to an extent greater than that of a corresponding dideoxynucleotide.
20. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent of at least, approximately, two-fold greater than incorporation of a corresponding dideoxynucleotide.
21. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent at least, approximately, five-fold greater than incorporation of the corresponding dideoxynucleotide.
22. (presently amended) The method of claims 2 or 3 wherein the acyclonucleotide is incorporated to an extent at least, approximately, nine-fold greater than incorporation of the corresponding dideoxynucleotide.
23. (withdrawn) The method of claims 19 wherein the acyclonucleotide comprises ROX-acyclo-CTP and the dideoxynucleotide comprises ROX-ddCTP.
24. (withdrawn) The method of claims 23 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, two-fold greater than that of ROX-ddCTP.
25. (withdrawn) The method of claims 23 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, five-fold greater than that of ROX-ddCTP.

26. (withdrawn) The method of claims 23 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, ten-fold greater than that of ROX-ddCTP.

27. (presently amended) The method of claims 1-3 2 wherein the DNA polymerase is additionally thermostable.

28. (presently amended) The method of claims 1-3 2 wherein the DNA polymerase has no detectable exonuclease activity.

29. (presently amended) The method of claims 1-3 2 wherein the DNA polymerase has been mutated so as to have an exonuclease activity of less than about 5% of the exonuclease activity of the unmodified enzyme.

30. (presently amended) The method of claims 1-3 2 wherein the DNA polymerase has been mutated so as to have an exonuclease activity of less than about 25% of the exonuclease activity of the unmodified enzyme.

31. (presently amended) The method of claims 1-3 2 further comprising the step of employing the resulting sequence-specific termination product or products in DNA sequence determination.